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Optimization of growth medium for microbially induced calcium carbonate precipitation (MICP) treatment of desert sand

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Abstract: Wind-induced sand erosion is a natural process, and can have several negative impacts on human health, environment, and economy. To mitigate the wind-induced sand erosion, an environmental friendly technique that helps to bind soil particles is desirable. The microbially induced calcium carbonate precipitation (MICP) treatment has lately become renowned and a viable alternative to enhance the binding of sand particles (especially against wind erosion). The efficiency of *Sporosarcina pasteurii* bacteria in inducing calcite formation can be influenced by various factors, including the type of growth media used for bacterial culture. Most of the studies have mainly validated the efficiency of *S. pasteurii* bacteria usually under single growth media for the MICP treatment. However, the efficiency of *S. pasteurii* under different growth media on calcite formation is rarely explored. The current study explores the effect of *S. pasteurii* bacteria on calcite formation under the presence of three different growth media, namely, molasses (MS), tryptic soy broth (TB), and nutrient broth (NB). The three growth media have been applied in the laboratory with and without bacterial solution (control samples). Altered cementation media concentrations (0.5 and 1.0 M) with different pore volumes (PVs), namely, 0.25, 0.50, and 1.00 PV were used in sand-filled tubes for 7 and 14 treatment cycles (1 cycle=24 h). The pH and EC were measured for 12-h period in every 2 h interval, to monitor values at the time of treatment at room temperature. The calcite precipitation was confirmed using SEM (scanning electron microscope), PXRD (powder X-ray diffraction), and calcimeter tests. It was observed that MS generates lower calcite precipitation as compared with NB and TB. However, MS has the advantage of being more economical and abundant (waste product from sugar mills and refineries) as compared with other growth media (NB and TB). It was observed that the minimum and the maximum calcite precipitation using MS is 5% and 12%, respectively. The findings using MS in the present study was compared with the literature and found that precipitation of calcite using MS is effective to stabilize soil against wind erosion.

Keywords: growth media; molasses; tryptic soy broth; nutrient broth; *S. pasteurii*; calcium carbonate

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1 Introduction

Deserts are amongst the most extreme and inhospitable ecosystems on the Earth, covering around one-third of the Earth's land surface. For example, In India, around 32×10^6 hm^2 of area is covered by arid and semi-arid regions. Wind-induced sand erosion is a natural process that occurs in these

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regions, where strong winds carry sand particles and deposit them in other areas. However, when it occurs at an excessive rate, it can have several negative impacts on human health, environment, and economy (Dagliya et al., 2022a). Wind-induced sand erosion can also cause pollution by depositing sand particles on roads, buildings, and other infrastructure, reducing visibility and causing accidents. The sand can also damage crops and livestock, reducing agricultural productivity and food security. Sand dunes are particularly vulnerable to erosion, and their destabilization can cause land degradation and loss of habitat. Mitigation of sandstorms and restricting land degradation are global challenges (Miao et al., 2020).

The typical methods of preventing wind erosion such as vegetation, sand hurdles, blockades, chemical equilibrium, and engineering measures are probable to become inefficient over a period (Goudie and Middleton, 2006). In comparison to existing ground improvement techniques, namely, using farming residue, industrial waste, and fibers, the microbially induced calcium carbonate precipitation (MICP) has recently developed as a strong approach for reinforcing ground particles (Chou et al., 2011; Sun et al., 2019; Sharma et al., 2021a).

The MICP is developing as an efficient concentration enrichment technique both for sand and clay (Sharma et al., 2021b; Tiwari et al., 2021). *Sporosarcina pasteurii* is one of the eminent ureolytic microbial species capable of inducing $CaCO_3$ deposition via the MICP activity for a variety of engineering applications (Davood et al., 2022) by causing metal ions to bond with acid radical ions. One amongst the popularly exercised techniques to produce carbonate precipitation is the hydrolysis of urea by the addition of extremely active urease-producing bacteria (such as *S. pasteurii* and *Bacillus megaterium*) (Sharma et al., 2020). The MICP is still in debate in terms of role of the bacteria. Research has been performed using microfluidic chip to quantify calcite formation, their distribution, and the rate of growth (Xiao et al., 2021a). Also the MICP bonds were examined with experimentations at the grain range (Xiao et al., 2022a). The MICP estimated costly for large-scale deployment due to growth media utilized in the biotechnological procedure for bacteria cultivation. Although the NB (nutrient broth) consumed in the MICP process is high-priced, however, by tuning its cementation and nutrition solutions, the cost of this approach can be minimized. A few studies have employed substitutes for NB, such as maize steep alcohol, vinasse, and syrup, which resulted in a cost effective bio-treatment (Maleki et al., 2016; Nikseresht et al., 2020).

Due to its inherent accessibility, effectiveness, and firmness, the MICP is presented as a highly desired method. *S. pasteurii* is used in the MICP, which hydrolyzes urea to produce $CaCO_3$ deposits that clog soil pores (Nasir et al., 2022). The microorganisms (*S. pasteurii*) utilize the carbon source from the growth media to produce enzymes that break down urea, which results in the production of ammonium ions (NH_4^+) and carbonate ions (CO_3^{2-}). The CO_3^{2-} then react with the calcium ions (Ca^{2+}) to form $CaCO_3$ (Xiao et al., 2020; Wu et al., 2023). Figure 1 shows the MICP mechanism.

Overall, the role of the growth media is to provide the microorganisms with necessary nutrients to carry out the precipitation process effectively, leading to the formation of a solid matrix that improves the durability and strength of sand. The efficiency of *S. pasteurii* in calcite formation can vary depending on the growth media used, as different media provide different nutrients and conditions for bacterial growth. It was also observed by Jiang et al. (2016), the rate of urea decomposition was slightly influenced by oxygen availability.

In the current study, different growth media, i.e., molasses (MS), tryptic soy broth (TB), and NB have been used to assess the optimal growth of bacteria and its feasibility to serve as a suitable alternative media for bacterial growth, urease activity, and calcium carbonate precipitation (Omoregie et al., 2019). The types of growth media selected here are on the basis that they must impart all the nutrients imperative for life and growth of bacteria. The purpose of the present study is to explore the influence of different growth media on *S. pasteurii* cultivation with different concentrations and pore volumes (PVs), and hence, the formation of calcite in the MICP treated soil.

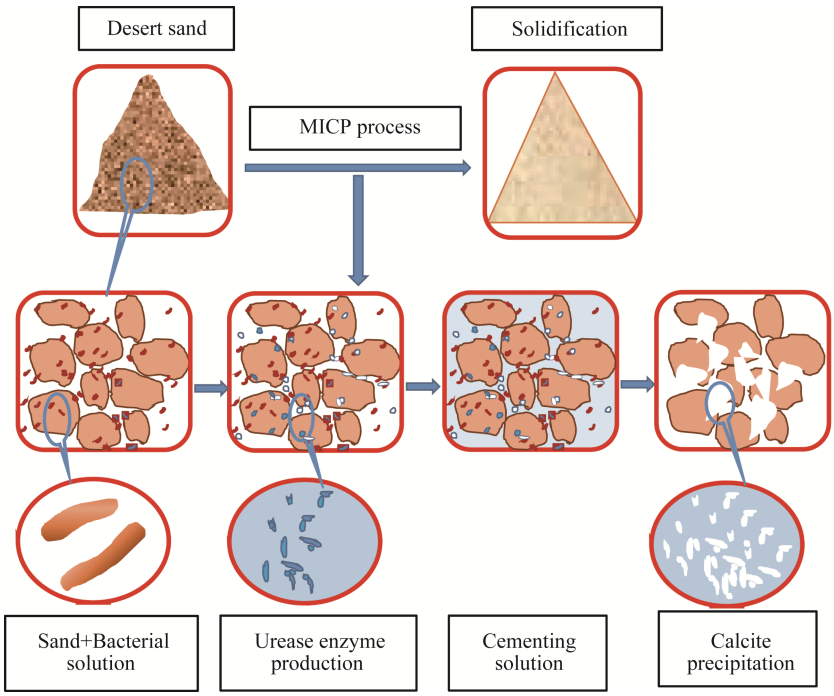


Fig. 1 Mechanism of *Sporosarcina pasteurii* precipitation in desert sand. MICP, microbially induced calcium carbonate precipitation.

2 Materials and methods

2.1 Study area and soil sampling

The sand sample was collected from Tinwari Village of the Rajasthan Province located in the western India. Figure 2 shows the particle size distribution curve of the soil sample. Table 1 summarizes the characteristics of the sand sample. As shown in Table 1, the optimum moisture content and the maximum dry density (MDD) is 12.70% and 1.65 g/mL, respectively.

2.2 Different growth media

The growth media used to culture *S. pasteurii* can significantly affect the bacterial growth rate. Three different growth media namely, NB, TB, and MS have been used in present study to analyze calcite formation for binding of particles. The selection of growth media (NB and TB) has

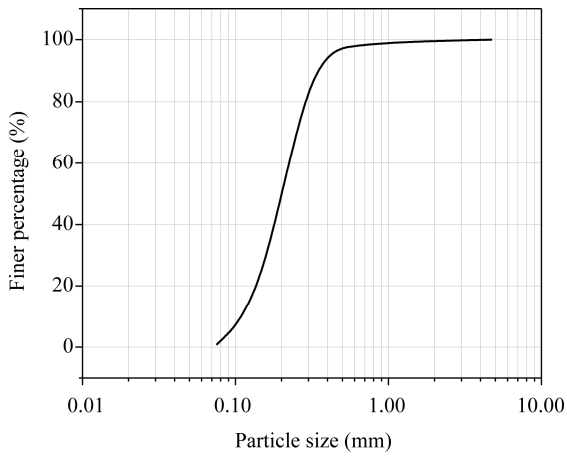


Fig. 2 Particle size distribution curve for sand sample (Dagliya et al., 2022b)

Table 1 Basic properties of desert sand

C_u (mm)	C_c (mm)	D_{10} (mm)	D_{30} (mm)	D_{60} (mm)	D_{50} (mm)	OMC (%)	MDD (g/mL)	G	e_{max}	e_{min}
1.83	1.09	0.13	0.18	0.23	0.21	12.70	1.65	2.57	0.90	0.62

Note: C_u, uniformity coefficient; C_c, coefficient of curvature; D_{10}, 10% of the particles are finer than this size; D_{30}, 30% of the particles are finer than this size; D_{60}, 60% of the particles are finer than this size; D_{50}, mean size of particles; OMC, optimum moisture content; MDD, maximum dry density; G, specific gravity; e_{max}, maximum void ratio; e_{min}, minimum void ratio.

been performed on the basis of previous studies on the MICP, and MS has been selected as growth media, as it is waste product, available freely, and fulfill requirement of bacterial food. NB and TB are not waste product like MS, rather they are commonly used laboratory reagents. NB is a general-purpose growth medium that provides a source of carbon, nitrogen, and other essential nutrients required for bacterial growth. *S. pasteurii* grown in NB has been shown to efficiently induce calcium carbonate precipitation. TB is a rich medium that contains a mixture of soybean meal and trypticase, which provides nitrogen and carbon sources. Tryptic soy broth is a versatile culture medium that can be used to grow a wide range of microorganisms, including yeast, mould, and common facultative anaerobic, aerobic, and lactic bacteria. Molasses is one of the precious byproduct of sugarcane. About 1 t of sugarcane produces 4% of molasses in sugar processing industry, which is available freely as waste residue. Molasses contains rich source of nutrients, and is employed as an effective raw material for the production of organic acids especially ethanol (Devi et al., 2019). Molasses application improves soil qualities by enhancing particle adhesion, allowing for the establishment of a strong inter particle link, which increases the stability of sand particles.

2.3 *S. pasteurii* microbe solution

The bacterial strains *S. pasteurii* were stored at −20°C before use. The bacterial culture (refer to Fig. 3a–c) was made by means of 3 types of bacterial growth media, MS (200 mL/L), NB (25 g/L), and TB (5 g/L). Autoclaving has not been performed to match the field condition. Inoculation of bacteria has been conducted in laminar air flow cabinet. In order to initiate bacteria growth, we kept inoculated bacteria solutions in an orbital shaking incubator for 1 d at a constant temperature of 30°C and rotation pace of 200 r/m under aerobic conditions (Fig. 3d) (Dagliya et al., 2022c). A spectrophotometer operating at a wavelength of 600 nm was used to measure the optical density (OD) of the pure bacterial solution (Fig. 3e). The noted down values were 1.025, 0.950, and 1.232 for NB, TB, and MS, respectively.

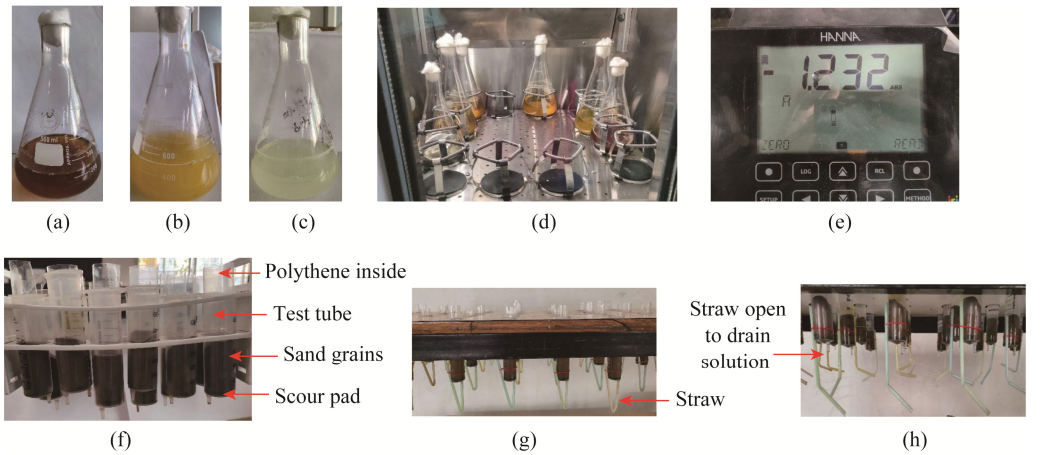


Fig. 3 Systematic diagram for the preparation of bacterial solution, treatment process, and testing kit. (a), bacterial culture MS (molasses); (b), bacterial culture NB (nutrient broth); (c), bacterial culture TB (tryptic soy broth); (d), orbital shaking incubator; (e), spectrophotometer; (f), treatment setup; (g), tubes with cementation solution; (h), drain the cementation solution.

2.4 Cementation media

Chemicals such as urea, ammonium chloride, calcium chloride dihydrate, growth medium, and sodium bicarbonate were all included in cementation media, which was used to hydrolyze urea. Table 2 summarizes the ingredients and their content for 0.5 and 1.0 M concentration. Mixture contains permutations of congregation of calcium chloride dehydrate and urea with different PVs (Table S1). It should be noted that for 1.0 M concentration, the amount of calcium chloride dehydrate is twice that for 0.5 M solution.

Table 2 Typical ingredients for cementation solution in 0.5 and 1.0 M concentrations

No.	Ingredient	0.5 M	1.0 M
1	Nutrient broth (NB; g/L)	3.00	3.00
2	Tryptic soy broth (TB; g/L)	2.50	2.50
3	Molasses (MS; mL/L)	100.00	100.00
4	Urea (mL/L)	30.03	30.03
5	Calcium chloride dehydrate (mL/L)	36.75	73.50
6	Sodium bicarbonate (mL/L)	2.12	2.12
7	Ammonium chloride (mL/L)	10.00	10.00

2.5 Treatment procedure

Cylindrical plastic vessel (tube) of 60 mL capacity containing polythene and scouring pad at the bottom was filled with 50 g of desert sand. The sand was filled with the help of a revolving cone to attain an approximate relative density of 50%. Straw was used to drain and undrain the solution (Fig. 3g and h). Bacterial solution using different growth media (1.0 PV for each tube), which was prepared without any sterilization process was poured into the plastic tube and left for 24 h (termed as attachment duration). After that cementation solutions (without calcium chloride dehydrate) in different PVs were poured and again left for 24 h (termed as simulation duration). Regular cementation solution was poured after 24 h and left for 24 h (termed as 1 treatment cycle). Before starting next cycle, cementation solution was drained by opening straw. Procedure is repeated for 7 cycles and 14 cycles with three growth media and different groupings of urea–calcium chloride dehydrates in conjunction with the regulators.

2.6 pH and electrical conductivity (EC)

The values were recorded during bio-cementation process. Values of pH and EC were noted using pH meter and conductivity analyzer, respectively. The readings were recorded for 1.0 and 0.5 M solutions with and without bacterial solutions. The readings were obtained for 12 h in every 2 h interval (Tiwari et al., 2021) at a known temperature. The laboratory temperature recorded during the treatment was between 12°C and 14°C.

2.7 Calcite content and micro-characterization of biotreated samples

After completing treatment cycles, solutions were drained out and the samples were left for 24 h. Then all samples were heated for 24 h in the oven at 60°C to drag wet sample out of tube without being ruptured. The samples were then collected and kept at 105°C (in an oven) for up to 24 h. Evidently, there was more calcite precipitation in the top layer due to pouring of chemicals. Subsequently, top 5 mm layer of each sample was removed. Exposed layer of the sample was then collected and ground into powder. Calcimeter-pressure guage from the Fann Instrument Company (Model 432, Fann Instrument Co., Houston, USA) was adopted to determine the percentage of calcite precipitation. During the calcite test, the pressure inside the calcimeter cylinder rose, when HCl was applied to the biotreated sand in the device. The amount of calcite precipitation involved is dependent on the enhanced pressure, which was detected using a pressure gauge. Calcite precipitation was also estimated using SEM test (schottky field emission scanning electron

microscope of JOEL JSM-7610F PLUS, JOEL Ltd., Tokyo, Japan) and PXRD (powder X-ray diffraction) test (automated multipurpose X-ray diffractometer of Rigaku Smart Lab., Tokyo, Japan), which were conducted in laboratory at IIT (Indian Institute of Technology) Indore, India. The gold sputter-coated sub-samples were subjected to SEM analysis, and SEM images were collected at different magnifications at 15 kV to ascertain the existence of calcite crystals and microbial beds. While PXRD analysis was adopted to determine the type of crystal structure and presence of minerals in biotreated sand samples. This study was carried out in the 20°–90° scanning range.

3 Results

The optimization of growth media in the MICP process for the formation of $CaCO_3$ has been extensively examined through several tests, namely calcite precipitation, SEM, and PXRD. The formation of $CaCO_3$ depending on pH and EC of the cementation media for the hydrolysis of urea was also measured.

3.1 EC and pH analysis

This section shows chemical alternation with the MICP treatment with different growth media to represent test results with appropriate reasoning for the formation of $CaCO_3$ in soil matrix. Figure 4 shows the EC experimental results of treated samples, with three different growth media and dual cementation media concentrations (with bacteria). All the biotreated samples exhibited a rising EC trend over the time, and the maximum values were attained between 6 and 8 h. The maximum values of EC with bacterial solution for S1.0NB, S1.0TB, and S1.0MS were 101.00, 92.69, and 72.00 mS/cm, respectively, and for S0.5NB, S0.5TB, and S0.5MS, the EC values were 76.99, 83.05, and 54.11 mS/cm, respectively. The rate of urea hydrolysis was higher in samples of cementation media concentrations of 1.0 M as compared with that in 0.5 M (Fig. 4a). Figure 4b showed the experimental results of EC with three growth media and dual cementation media concentrations for control samples. It was analyzed that EC values were less in control sample compared with solution that was prepared with bacterial solution. Analysis of Figure 4a and b showed that the presence of bacteria enhanced the speed of urea hydrolysis and contributed in production of amount of calcite precipitation.

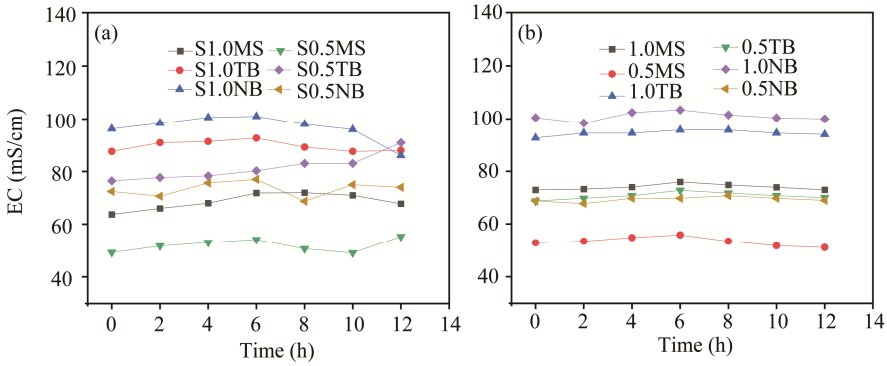


Fig. 4 Electrical conductivity (EC) for three different growth media and dual cementation media concentration (1.0 M and 0.5 M). (a) bacteria; (b), without bacteria. S, *Sporosarcina pasteurii*; MS, molasses; NB, nutrient broth; TB, tryptic soy broth.

pH is also a key element that controlled the pace of reactions. Deviation in pH with different cementation media concentrations and type of growth media has been shown with presence of bacteria (Fig. 5a) and with control sample (Fig. 5b). The maximum values of pH with bacterial solution for NB, TB, and MS were 7.9, 7.7 and 6.9, respectively. Initially, pH value increases and stabilized after 6–8 h, also 0.5 M cementation solution has a lower pH compared with 1.0 M cementation solution.

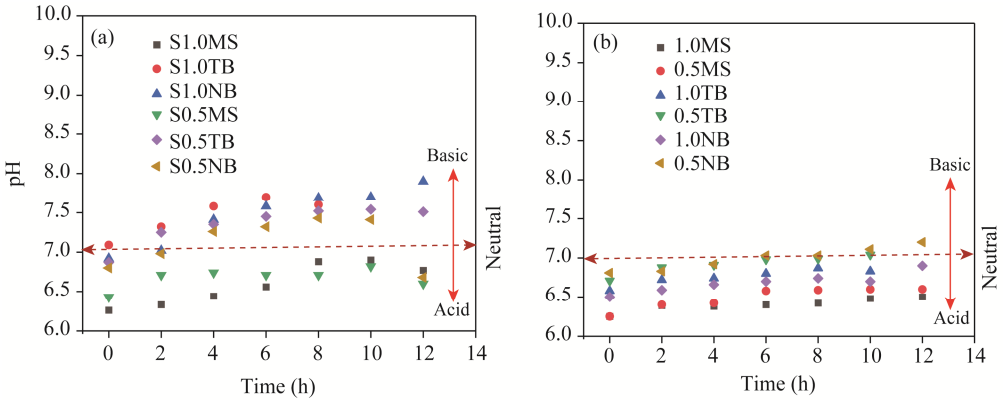


Fig. 5 pH for three different growth media and dual cementation media concentration (1.0 M and 0.5 M). (a) bacteria; (b), without bacteria. S, *Sporosarcina pasteurii*; MS, molasses; NB, nutrient broth; TB, tryptic soy broth.

3.2 Calcite precipitation analysis

The MICP treatment of desert sand was executed for 7 and 14 cycles with different growth media. Various concentrations of growth media with and without bacteria were used to treat the samples in tubes. Samples injected with bacteria showed the calcite precipitation caused by the microorganism over time. The specimen from the top portion of the cylindrical plastic vessel was obtained for calcite content analysis after the upper 5 mm layer of calcite was removed. Figure 6a shows the variation in $CaCO_3$ percentage for three different growth media, 0.5 M cementation solution, with and without bacteria solution, and different PVs. Figure 6b shows the variation in percentage calcite precipitation for three different growth media, 1.0 M cementation solution with bacteria solution, and different PVs. It was observed that the maximum $CaCO_3$ percentages with 1.0 M cementation solution, 1.00 PV, and 14 d treatment for NB, TB, and MS were 31%, 21%, and 12%, respectively, and with 0.5 M cementation solution, they were 29%, 25%, and 11%, respectively. On the other hand, the minimum $CaCO_3$ percentages with 1.0 M cementation solution, 0.25 PV, and 7 d treatment for NB, TB, and MS were 10%, 9%, and 7%, respectively, and with 0.5 M cementation solution, they were 8%, 7%, and 5%, respectively. It was noted that S1.0NB1.00PV14D imparted the highest calcite formation, and S0.5MS0.25PV7D reported the lowest calcite formation.

The soil specimen from the downward section of the cylindrical plastic vessel exhibited very

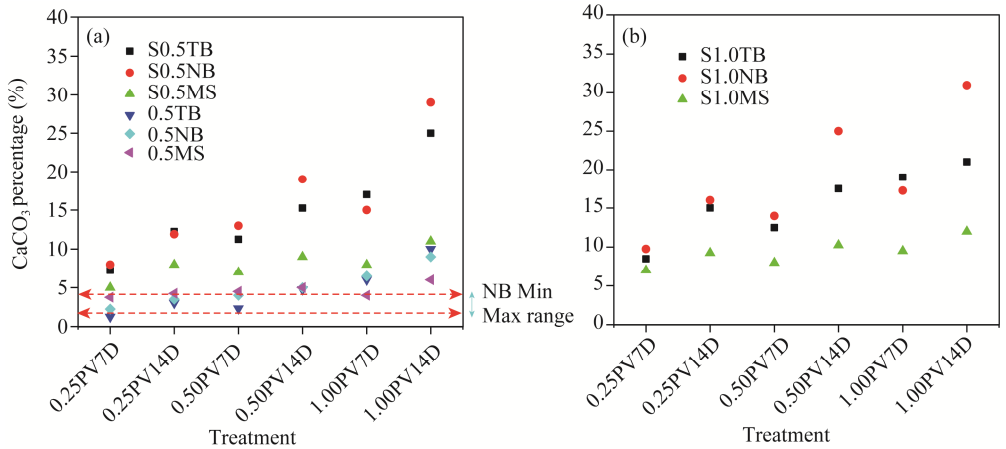


Fig. 6 Variation in $CaCO_3$ percentage for different growth media with and without bacteria solution, different pore volumes, and treatment days. (a), 0.5 M cementation solution; (b), 1.0 M cementation solution. S, *Sporosarcina pasteurii*; MS, molasses; NB, nutrient broth; TB, tryptic soy broth; PV, pore volume; D, treatment days.

little calcite precipitation, which was between 2% and 3% only, due to bio clogging primarily in the top 1.0–1.5 cm layer. Despite the homogeneity and calcite bond potency being significantly excessive in the 14 cycles treated samples, 1.0 M concentration samples of all three-growth media demonstrated adequate outcomes post 7 and 14 cycles of treatment.

It was observed from the results that MS displayed lower calcite formation compared with NB and TB. MS media gave 5% minimum $CaCO_3$ percentage for 0.5 M and 0.25 PV in 7 cycles and 10% maximum $CaCO_3$ percentage for 0.5 M and 1.00 PV for 14 cycles. Figure 6a showed that the minimum $CaCO_3$ percentage was 2% in 5 d and the maximum $CaCO_3$ percentage was 4% after 20 d treatment for scenario NB with 0.5 M cementation solution and 0.25 PV. Although calcite formation was lower as compared with the other two growth media, still this amount of calcite percentage was sufficient in stabilizing desert sand.

3.3 SEM analyses of samples treated with different growth media and concentration

The calcite crystal formed, and microbe bed was visible in the SEM images of bacteria and all the cementation media concentrations (Fig. 7a–d). SEM images of biotreated sand samples were taken at ×5000, ×20,000 or ×25,000 magnifications and 1 μm scale. More calcite crystals may be witnessed in SEM images obtained at various amplifications of 1.00 PV treated materials. Based on SEM analyses, the calcite precipitations were visible in all the combinations. As compared with the samples treated for 7 d, samples treated for 14 d seems to have more calcite crystals. All three types of growth media treated with a solution of 0.5 M concentration for 7 d showed uniformity and more precipitation. It is known that SEM images have limitations as it is merely a qualitative approach. More interpretation about calcite precipitation can be deduced from PXRD analyses, which is discussed in the next section.

3.4 PXRD analysis

PXRD analysis was conducted on samples treated with cementation medium at 0.5 M and 1.0 M concentrations using all three growth media. Rhombohedral calcite crystals were visible in all types of growth media in the PXRD data of 7 and 14 d treated samples. The lack of calcite in the sand prior to treatment was revealed using PXRD study of untreated sand (Fig. 8a). It could be inferred that initially desert sand was free of calcite with presence of only quartz in it. The proportional graphical scrutiny of treated samples with three growth media, two cementation solutions (0.5 M and 1.0 M), and with and without bacteria are shown in Figure 8b–g for 7 and 14 treatment cycles. The crest of calcite and quartz were observed in graphs using PXRD analyses of the treated samples.

4 Discussion

Present study deals with optimization of different growth media with *S. pasteurii* bacteria using different PVs to find feasible solution, so that the MICP can be used economically. The efficiency of *S. pasteurii* bacteria in calcite formation has been confirmed by calcite precipitation, SEM, and PXRD test. From the present study, MS growth media can be used economically to stabilize desert soil, as for desert soil stabilization only top layer treatment is required to bind particles.

The pH and EC can both affect the pace of urea hydrolysis. Maintaining the optimum pH range and controlling EC of the solution can help to optimize the rate of urea hydrolysis in various MICP applications. There was correlation between calcite formation, and it was observed that calcite formation increases with the increases in EC value. Similar results of EC with NB has been obtained by van Paassen (2011). EC values were higher with presence of bacteria compared with control sample (Li et al., 2022), also higher with higher molar concentration. The results were confirmed with studied by Sharma et al. (2021c). The transformation of non-ionic compounds to ionic compounds leads to the conclusion that EC value of the cementation media solution increased in proportion to rise in ions concentration and resulted in higher calcite formation (Tiwari et al., 2021). The minimal discrepancy may be due to the temperature and the pace of bacterial growth, since experimental results and anticipated data were nearly identical.

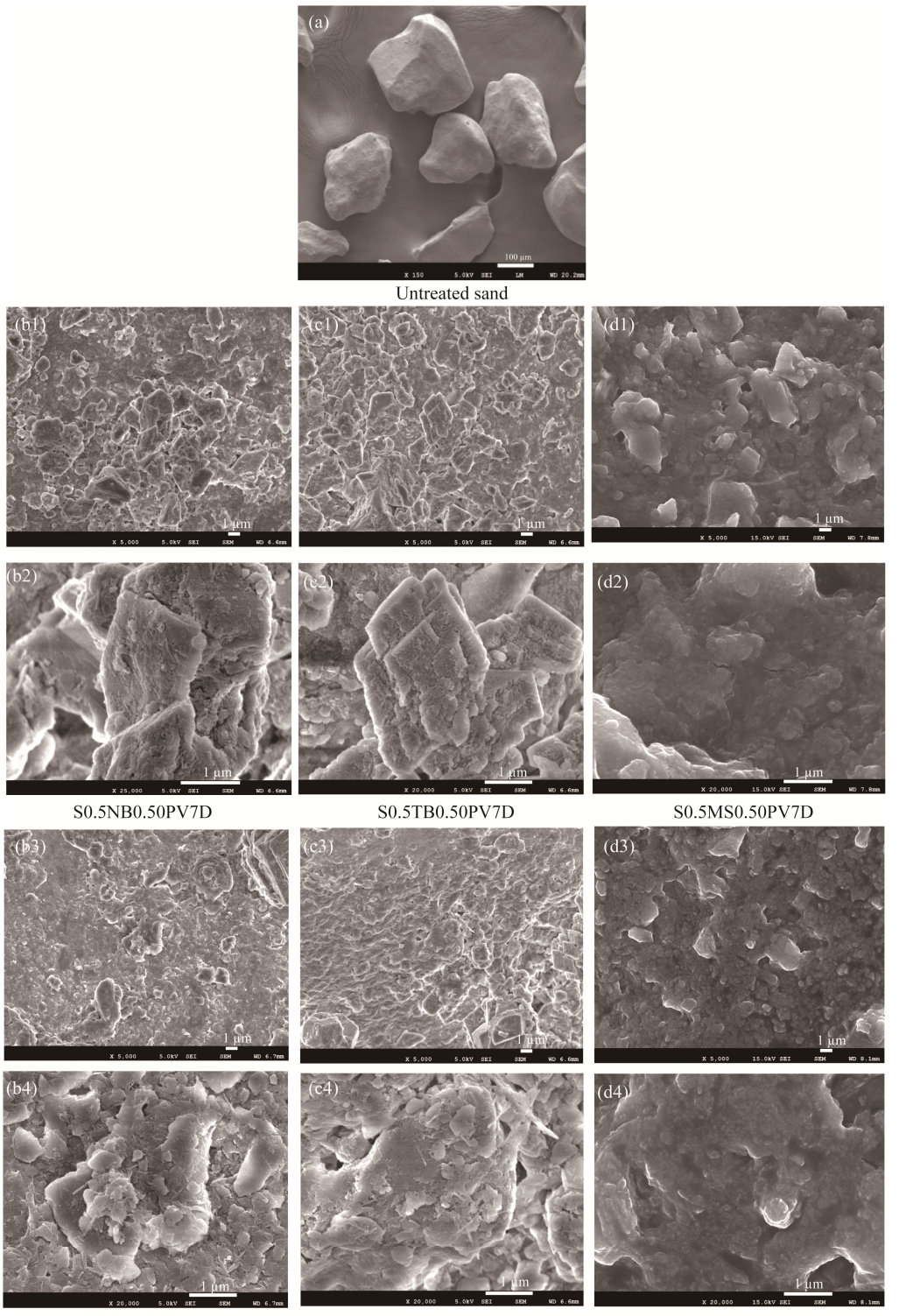
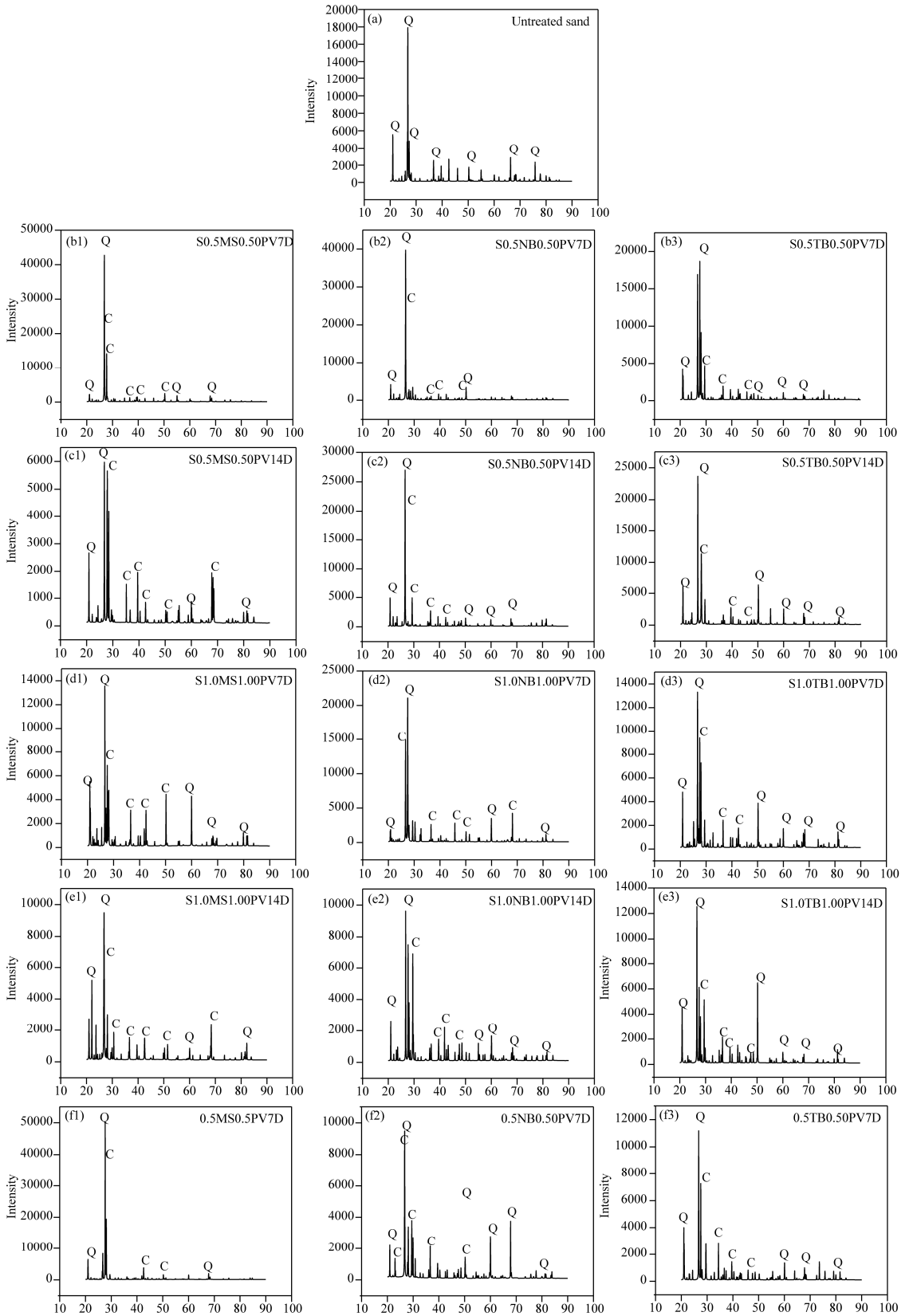


Fig. 7 Scanning electron microscope (SEM) images at different magnifications of soil samples treated with different pore volumes and growth medias after 7 d of curing. (a), untreated sand; (b1–b4), NB with 0.5 M and 0.50 PV or 1.0 M and 1.00 PV; (c1–c4), TB with 0.5 M and 0.50 PV or 1.0 M and 1.00 PV; (d1–d4), MS with 0.5 M and 0.50 PV or 1.0 M and 1.00 PV; (b1–d1, b3–d3), ×5000 magnification; (b2–d2, b4–d4), ×20,000 or 25,000 magnification; S, *Sporosarcina pasteurii*; NB, nutrient broth; TB, tryptic soy broth; MS, molasses; PV, pore volume; D, treatment days.



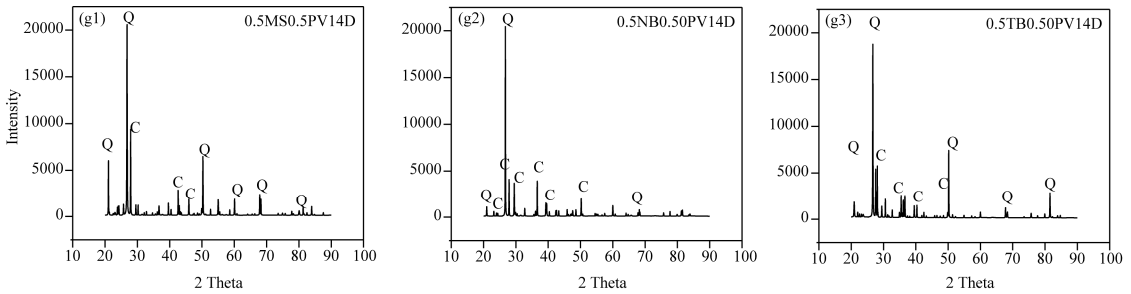


Fig. 8 Powder X-ray diffraction (PXRD) analysis under different pore volumes (PVs) and growth medias for 7 and 14 d. (a), untreated sand; (b1–b3), 0.5 M, 0.50 PV, and 7 d with bacteria; (c1–c3), 0.5 M, 0.50 PV, and 14 d with bacteria; (d1–d3), 1.0 M, 1.00 PV, and 7 d with bacteria; (e1–e3), 1.0 M, 1.00 PV, and 14 d with bacteria; (f1–f3), 0.5 M, 0.50 PV, and 7 d without bacteria; (g1–g3), 0.5 M, 0.50 PV, and 14 d without bacteria; S, *Sporosarcina pasteurii*; MS, molasses; NB, nutrient broth; TB, tryptic soy broth; PV, pore volume; D, treatment days; C, calcium carbonate; Q; quartz.

Temperature played an important role for EC measurement, as urease activity changes with temperature (Xiao et al., 2021b). In the present study, NB treated samples showed the highest EC values, followed by TB treated samples, and then MS treated samples. Therefore, the pace of urea hydrolysis can be determined using the expected values of EC, particularly for a given cementation solution concentration and types of growth media (Wu et al., 2011). According to Liu et al. (2021), the inserted bacteria may be unclean because of the existence of extra strain or may not have survived due to warmness (the operating heat range was 25°C–60°C) (Whiffin, 2004). Biogeochemical reactions in the MICP process generally raise the EC due to the hydrolysis of urea and the transformation of non-ionic molecules to ionic compounds. The changes in EC values can therefore be used to estimate the pace of hydrolysis of urea or urease activity.

Calcite formation also has variation with pH values, in the starting, and pH value increased quickly due to the biogeochemical reactions (Omoregie et al., 2019), but stabilized with time. Owing to the production of ammonia gas and the subsequent synthesis of ammonia byproducts, pH initially increased rapidly (van Paassen, 2009; Sharma et al., 2021c). A similar rise in pH was also observed in study by Choi et al. (2017), Kim et al. (2018), and Sharma et al. (2021c). Goodarzi et al. (2016) studied that shear strength of soil increases with increase in pH value. Increase in calcite formation inside soil pores has also been observed. Temperature of the surrounding environment and the length of the reaction seem to influence calcite precipitation (Nikseresht et al., 2020). This is because they have an impact on processes, including microbial activity/growth, urease activity, and solubility of $CaCO_3$. Temperature and pH have a complicated effect on the MICP. Increased alkalinity and pH encouraged $CaCO_3$ precipitation.

The present study clearly demonstrated that the precipitation rate was excessive in NB samples with 1.00 PV as compared with TB and MS. The release of ammonium as ammonia gas in the air was ascribed to the larger molarity of urea than $CaCl_2{\cdot}2H_2O$, which increased pH and reaction rate (Zhao et al., 2016). In comparison to samples with 0.5 M and 1.0 M concentrations, the rate of precipitation was significantly high in 1.0 M, because bacteria performed as a nucleation site at higher pH levels (Riveros and Sadrekarimi, 2020). The findings of the current research are reliable with that of Nikseresht et al. (2020). $CaCO_3$ precipitation is the only reason that outcomes in the formation of soil surface that provides resistance against penetration and strength (Mahawish et al., 2018). Bio clogging occurred at the surface or close to the pouring point as a result of the faster reactivity and precipitation rate. It was analyzed by Gu et al. (2018) and Xiao et al. (2021b, 2022b), which bio clogging occurred in the top of soil sample and precipitation of $CaCO_3$ was not uniform towards bottom of the sample. Neither a very high nor a very low rate of ureolysis appreciated. A lower rate of urea hydrolysis lengthened the time for treatment to produce an adequate volume of $CaCO_3$ precipitation, whereas a higher rate encouraged $CaCO_3$ formation close to the pouring point.

The formation of calcite crystal was confirmed through SEM images. Existence of microbe beds, which appeared as voids, on the overlay of calcite crystals, has been confirmed by Nasir et

al. (2022) and Naeimi et al. (2023). Calcite crystals of varied sizes were produced by the bacteria and different cementation media combinations. By impression of bond among bacteria and cementation media, the variety in crystal sizes was linked to conflicts between crystal development and production of fresh crystals. This is because the development rate of present crystals was slowed by the production of new crystals as result of clashing (Gandhi et al., 1995). Due to high cementation media concentration, the reaction rate of urea hydrolysis was high, which favored the production of fresh calcite crystals over the development of present crystals. The calcite picks were also confirmed with the PXRD test, which was similar with Nasir et al. (2022).

5 Conclusions

Present study compares the influence of MS growth media on calcite formation in comparison with other commonly adopted growth media (NB and TB). We found that small-scale bacterial treatment of desert sand was performed in plastic cylindrical mold to infer the calcite formation based on varied growth media, cementation media concentrations, and PVs. The interpretation of measured calcite content was carried out using calcimeter test and micro characterization test, namely PXRD and SEM. Following inferences can be illustrated through the current study. All three growth media were observed in terms of calcite formation. Although MS had less calcite formation over NB and TB, but calcite percentage was sufficient and made it optimal for desert sand stabilization as a growth media. Moreover, in stabilization of desert sand, only top layer needs to be stabilized for its protection from wind induced sand erosion. Hence, MS can be considered as optimal growth media for the MICP treatment of desert sand stabilization. The rate of ureolysis was high in 1.00 PV and 1.0 M cementation solution, as compared with 0.50 PV, 0.25 PV, and 0.5 M cementation solutions. The sample has excessive precipitation in 14 treatment cycles, to the topmost layer of the treated soil. About twice calcite precipitation was observed, when samples were treated for 14 treatment cycles instead of 7 treatment cycles with same cementation media solutions. The growth of calcite crystals and peaks of calcium enhanced with the number of treatment cycles. And molar concentration and PV played crucial roles on calcite formation.

6 Limitation and future scope

Largely, the study exhibited the viability of the MICP on desert sand using different growth media. The purpose of the study was to optimize the growth media using MS, for precipitation of $CaCO_3$. $CaCO_3$ precipitation depends on many factors including temperature. In the present study, temperature effect has not been considered, it can be taken into account in the future scope of the work. Also uniformity of calcite formation, durability analysis, and large scale testing using MS need to be studied before applying it to the field.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Original content was written by the first author, who also carried out the laboratory tests. Control of student, research guidance, and concept development comes within the purview of the second author. Co-supervision of the first author and text revision fall within the purview of the third author.

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Appendix

Table S1 Testing plan summarizing treatment combinations with different growth mediums, cementation solutions, pore volumes, and duration

Sample designation	Cementation solution (M)		Growth media			Pore volume			Treatment days (d)	
	0.5	1.0	TB	NB	MS	0.25	0.50	1.00	7	14
S0.5TB0.25PV7D	√		√			√			√	
S0.5TB0.25PV14D	√		√			√				√
S0.5TB0.50PV7D	√		√				√		√	
S0.5TB0.50PV14D	√		√				√			√
S0.5TB1.00PV7D	√		√					√	√	
S0.5TB1.00PV14D	√		√					√		√
S1.0TB0.25PV7D		√	√			√			√	
S1.0TB0.25PV14D		√	√			√				√
S1.0TB0.50PV7D		√	√				√		√	
S1.0TB0.50PV14D		√	√				√			√
S1.0TB1.00PV7D		√	√					√	√	
S1.0TB1.00PV14D		√	√					√		√
S0.5NB0.25PV7D	√			√		√			√	
S0.5NB0.25PV14D	√			√		√				√
S0.5NB0.50PV7D	√			√			√		√	
S0.5NB0.50PV14D	√			√			√			√
S0.5NB1.00PV7D	√			√				√	√	
S0.5NB1.0PV14D	√			√				√		√
S1.0NB0.25PV7D		√		√		√			√	
S1.0NB0.25PV14D		√		√		√				√
S1.0NB0.50PV7D		√		√			√		√	
S1.0NB0.50PV14D		√		√			√			√
S1.0NB1.00PV7D		√		√				√	√	
S1.0NB1.00PV14D		√		√				√		√
S0.5MS0.25PV7D	√				√	√			√	
S0.5MS0.25PV14D	√				√	√				√
S0.5MS0.50PV7D	√				√		√		√	
S0.5MS0.50PV14D	√				√		√			√
S0.5MS1.00PV7D	√				√			√	√	
S0.5MS1.00PV14D	√				√			√		√
S1.0MS0.25PV7D		√			√	√			√	
S1.0MS0.25PV14D		√			√	√				√
S1.0MS0.50PV7D		√			√		√		√	
S1.0MS0.50PV14D		√			√		√			√
S1.0MS1.00PV7D		√			√			√	√	
S1.0MS1.00PV14D		√			√			√		√

Note: All samples were prepared in three replicates. S, *Sporosarcina pasteurii*; TB, tryptic soy broth; NB, nutrient broth; MS, molasses; PV, pore volume; D, treatment days.